

Extracellular Lipid Droplets in *Idiosepius notoides*, the Southern Pygmy Squid

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Abstract. Cephalopod metabolism typically involves carbohydrates and proteins, so that the lipid content of the mantle and all internal organs except the digestive gland is very low. Despite clear evidence of nonlipoid metabolic trends in cephalopods, we observed extracellular spheres, or droplets, in the cecum and digestive gland of newly collected juvenile, male, and female individuals of *Idiosepius notoides*, the southern pygmy squid. Prior to staining, the droplets were various shades of yellow and were often large enough to detect at 7× magnification. The droplets were less dense than water, hydrophobic, and sudanophilic, staining positively with Sudan III, Sudan IV, and Sudan Black B. We conclude that these spheres are lipid and that they derive from the squid's normal field diet. When newly collected squid were starved in the laboratory, the droplets disappeared in 7–8 d and then reappeared in the cecum about 3 h after feeding.

Introduction

Although cephalopods require considerable energy for rapid movement, growth, and reproduction, they apparently have limited capacity to metabolize or store lipids (Hochachka *et al.*, 1975; Storey and Storey, 1983; O'Dor and Webber, 1986; Moltschaniwskyj and Semmens, 2000). In 1975, cephalopod metabolism was described as poorly understood, and squid storage substrates were "a well known mystery in marine biochemistry" (Hochachka *et al.*, 1975). That mystery arises because lipids, efficient energy storage molecules due to their high per-gram energy content, are typically not abundant in cephalopods (Hochachka *et al.*, 1975; Castro *et al.*, 1992; Clarke *et al.*, 1994).

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Abbreviation: ML = mantle length.

Despite evidence of limited lipid metabolism in cephalopods, lipid storage has been reported for the digestive gland, the only cephalopod organ consisting of more than a few percent lipid. The cecum is not reported to be a typical lipid storage site, although cecal cells have been ascribed a variety of functions, including fat absorption in several species (Bidder, 1966; Boucaud-Camou and Boucher-Rodoni, 1983; O'Dor *et al.*, 1984; Westermann and Schipp, 1998).

Idiosepius notoides Berry 1921 is a small sepioid found in seagrass beds in southern Australian waters, from Cockburn Sound in Western Australia to Morton Bay, Queensland (Shepard and Thomas, 1989). We report the existence and retention of yellow spheres within the cecal lumen and digestive gland of field-collected specimens of *I. notoides* subjected to starvation, and explore the possible lipid nature of these droplets. We also speculate on the origin, function, and fate of the droplets.

Materials and Methods

Idiosepius notoides was collected by seining over seagrass beds (18 °C, 39 ppt salinity) near the mouth of the Port River, Adelaide, South Australia, on 29 March 2002 (Day 0). All squid were recognized by their small size (about 10 mm dorsal mantle length), by a pair of rounded fins near the rear of the body, and by their attachment to seagrasses and *Ulva* sp. using dorsal duoadhesive glands (Norman and Reid, 2000). Because idiosepiids are morphologically unusual, they historically have been placed with the teuthids but are currently classified as sepiids (Berry, 1932; Hylleberg and Natewathana, 1991); despite their cuttlefish alliances, they are commonly called pygmy "squid" and will be referred to as squid in this paper.

Squid that died on collection ($n = 2$) were examined on Day 0. Live squid were kept unfed in an aquarium connected to the recirculating seawater system (~16 °C, 40 ppt salinity, 12 h light:12 h dark cycle) until natural death or

sacrifice on Days 4–15. To investigate whether “starved” squid ate small organisms (e.g., copepods present in the recirculating seawater), three squid were videotaped individually (2.5 h total, 25 frames per second) in a tank measuring 19.3×7 cm (containing one liter of unfiltered seawater from the recirculating system).

Before sacrifice, each squid (chosen at random) was observed at about $10\times$ magnification in a small, flat dish for 15 min in an attempt to locate droplets prior to anesthesia or dissection. The dark-pigmented mantle of active, stressed animals often hid droplets. However, during the squids' occasional flashes of transparency, we could see through the mantle tissue and determine droplet location.

At sacrifice, squid were transferred (in seawater) to a freezer for terminal anesthesia, measured (dorsal mantle length = ML), and then decapitated (Boyle, 1991). (Although cold water may be analgesic rather than anesthetic [Boyle, 1991], in this paper the treatment is referred to as cold anesthesia.) The mantle was cut open to expose but not damage internal organs. We recorded gender, stage of sexual maturation (based on Lipiński's maturation scale as interpreted by Moltschaniwskyj, 1995), droplet presence, and sometimes droplet diameter. Droplets to be tested for lipid content were obtained by puncturing the cecum. Because it is unknown if handling of squid may break drops into smaller droplets (and we wanted larger drops for stain testing), we minimized handling of squid prior to anesthesia and during dissection.

Droplets were tested for lipid content using three stains: Sudan III, Sudan IV, and Sudan Black B (one stain per squid). Because these stains have very low water solubilities (ranging from <0.1 mg/ml for Sudan III to 0.7 mg/ml for Sudan IV; Green, 1990) compared to solubility in ethanol, staining solutions were prepared as saturated solutions in 70% ethanol. Staining solutions were prepared only a few days before use to avoid the deterioration that can occur in Sudan/alcohol solutions (Gurr, 1962).

We tried three approaches to cecal droplet staining (one method per squid). For ceca cut open unsubmerged, stain was pipetted directly onto the body. For ceca punctured submerged, filtered stain was added to the seawater (i.e., drops were stained floating on the water surface). Additional droplets collected from the water surface by patting with strips of filter paper were flooded with stain for about 10 min, followed by ethanol rinses to de-stain the paper. Control tests were conducted using food-grade vegetable oil. Positive stain for lipid includes yellow-orange for Sudan III, orange-red for Sudan IV, and blue-black for Sudan Black B (Conn, 1961; Gurr, 1962, 1965).

To determine if changes in extracellular lipid volume or location could be detected after feeding, we fed one squid and then repeatedly examined it for droplets. This squid (see 9.7-mm-ML male on Day 7, Table 1) was chosen because it seemed less stressed by handling: compared to most of our

other squid, its movements were less vigorous in the small observation dish, and it seemed to have a transparent mantle more frequently. No anesthesia was used on the day of feeding, but it had to be used on the second and third days after feeding. Before feeding, the squid had no cecal drops but did have two small, equal-sized drops at the anterior end of the digestive gland, one on the left and one on the right side. After it caught the live shrimp provided (*Hippolyte* sp., ~ 19 mm long), the squid was left undisturbed until it discarded the intact but almost empty exoskeleton 45 min later. No effort was made to locate oil droplets during feeding because preliminary work showed that disturbed squid tended to abandon their prey. We examined the squid for droplets immediately after its meal, at hourly intervals for the next 7 h, and then up to twice daily until no drops were detected at a magnification of about $30\times$. We measured the cecal droplets if the squid remained stationary and was transparent during observations.

To address the possibility of droplet expulsion, we kept four immature squid (4.4–6.9 mm ML) in individual glass bowls (colorless) until their oil droplets were undetectable. We used small bowls (100 ml of filtered seawater, 16°C) to decrease the surface area we would have to search for oil. These squid, from a separate collection made in late April 2002 near Noarlunga (south of Adelaide), all had oil drops when collected and when placed in the glass bowls. Prior to use in this experiment they were maintained in an aquarium connected to the recirculating seawater system and fed field-collected mysid shrimp for 1 week. After placement in individual dishes, these squid were kept without food and were examined with a dissecting microscope once per day to determine whether droplets were present in the digestive system. Prior to the daily cleaning and refilling of the bowls, the water surface was examined with a dissecting microscope for floating droplets and then was patted with white paper toweling, which was also examined for droplets.

Results

Of the 16 squid collected in March, 11 were males, 3 were juveniles, and 2 were females. Dorsal mantle length ranged from 6.5 to 16.5 mm. Females tended to be larger than males, as previously recorded (Norman and Reid, 2000); mantle length was 15.4 ± 1.1 mm (mean \pm SD) in females, 8.4 ± 0.9 mm in males, and 6.8 ± 0.3 mm in juvenile squid.

Droplets were easiest to locate in dissected, fresh squid or in healthy, stationary squid in transparent phase. Opaque mantle tissue in preserved and moribund squid hid the droplets. For active squid, it was difficult or impossible to obtain accurate droplet counts or measurements.

In the first week after collection, we examined one juvenile, nine male, and two female squid. Of these 12 un-fed squid, 11 had droplets (Table 1). Droplets were not detected

Table 1

Gender, reproductive maturity stage, and dorsal mantle length of starved *Idiosepius notoides* individuals examined Days 0–15 post-collection for extracellular droplets in the digestive tract

Days (post-collection)	Gender & maturity stage		Mantle length (mm)	Droplets seen?
0	J	1	6.5	+
0	M	3	9.0	+
4	M	3	9.1	+
4	M	3	8.7	+
4	M	3	8.1	+
4	F	5	16.5	–
5	M	3	8.0	+
5	M	3	8.0	+
5	M	3	8.0	+
7	M	5	9.8	+
7	F ^a	nd	14.3	+
7	M ^b	nd	9.7	+
9	J	1	nd	–
9	J ^c	1	nd	–
10	F ^a	nd	13.7	–
10	J ^c	1	7.0	–
14	M	3	8.6	–
15	M	3	6.5	–

J, juvenile; M, male; F, female; nd, no data.

^a Same squid examined Days 7 and 10.

^b This squid was used in a feeding experiment beginning later on Day 7.

^c Same squid examined Days 9 and 10.

in the largest squid, a female. During Days 9–15 after collection, we found no droplets in any of the unfed squid (Table 1).

Drops were most obvious in the cecum (Fig. 1a), a digestive sac near the rear of the body. The cecum wall was transparent, and the lumen of the organ contained a clear greenish or bluish fluid without obvious particles (Fig. 1a, b), all of which made the droplets conspicuous. We did not notice any distinct progression of color changes in cecal fluid (Lipiński, 1990). Cecal droplets moved around, apparently due to ciliary currents and cecal contractions. Because the drops floated, they were easily detectable in the cecum whether we removed a dorsal or ventral piece of mantle (Fig. 1a, b). Unlike the luminal oil droplets described in the loliginid squids *Septoteuthis lessoniana* and *Photololigo* sp. (Semmens, 1998), the droplets in *Idiosepius notoides* apparently are not membrane-bound; when pushed together with a microprobe, droplets could readily fuse.

We also saw droplets in the anterior end of the digestive gland, just under the edge of the uncut mantle, but these were often harder to detect than cecal drops. Drops were seen in the left, right, or both sides of the digestive gland simultaneously. We never found drops outside the cecum or digestive gland when we carefully cut only mantle tissue.

Droplet color varied among squid but typically looked like some shade of yellow. It was difficult to be sure of color

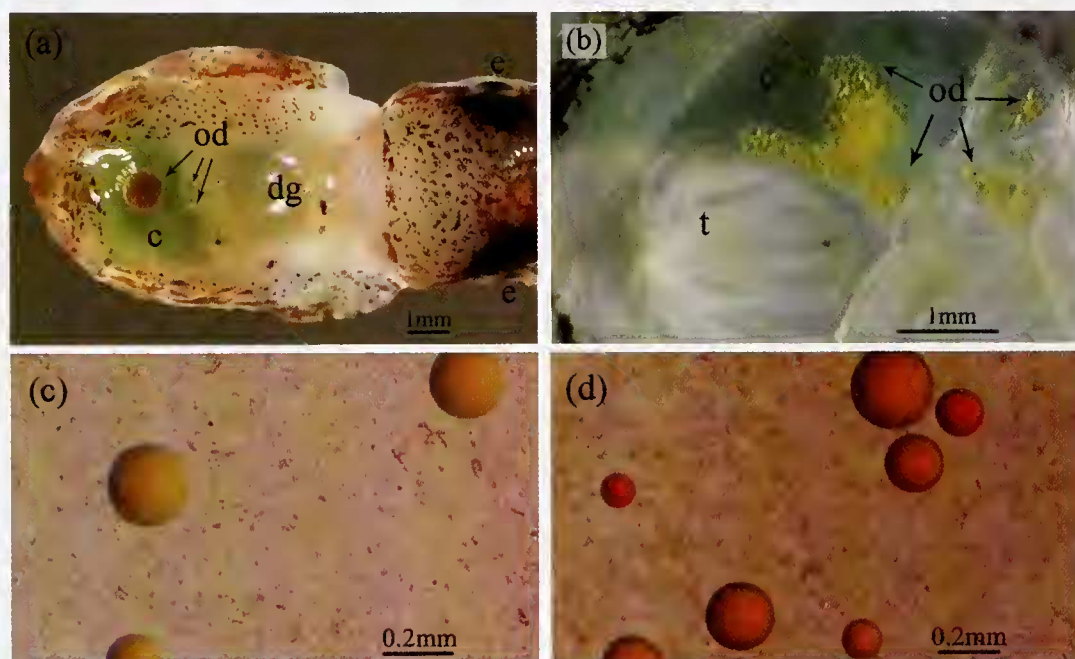


Figure 1. Cecal lipid droplets in *Idiosepius notoides*. Tissue was cut from dorsal (a) or ventral (b) mantle to expose the cecum and its yellow droplets. All oil drops shown are from starved male squid sacrificed Days 4–5 after collection (8–9 mm ML). The floating drops in c and d are from a cecum punctured under water. Filtered Sudan IV (in 70% ethanol) was added to the seawater. Photographs were taken every 5 min for 1 h on an Olympus DP10 digital camera attached to an Olympus SZH microscope. Shown are droplets at 5 min (c) and 60 min (d) staining. c = cecum, dg = digestive gland, e = eye, od = oil droplets, t = testis

comparisons for cecal droplets because the variable blue-green hues of the cecal fluid (e.g., Fig. 1a vs. 1b) probably altered our color perception. Larger drops of yellow food oils (almond, olive, sunflower, vegetable) all appeared colorless floating in seawater under the same lighting conditions. Unstained droplets collected onto white filter paper from two Port River squid were conspicuous at 10 \times due to their brilliant lemon yellow color, while droplets from three of the four Noartunga squid looked colorless in the cecum.

Number and size of droplets varied among squid. For example, one squid had a huge cecal drop (~0.9 mm in diameter; Fig. 1a), and another had dozens of tiny droplets (Fig. 1b). However, on Days 0–7 after collection, most starved squid had 2–14 droplets with a typical diameter of 0.05–0.2 mm per droplet.

Evidence supporting the lipid nature of the droplets is summarized in Table 2 and in Figure 1c and d. The yellow droplets were hydrophobic, typically forming small spheres in the cecum. When we cut a submerged cecum, droplets rapidly escaped and floated to the water surface. On hitting the air-water interface, larger drops "popped" from a sphere into a thin disk on the water surface. One large drop pipetted onto a piece of thin brown-paper bag and pressed onto the paper using Parafilm left a translucent window (11 mm in diameter) in the paper for a 48-h observation period, whereas other random fluids from the same squid did not. Floating droplets collected onto filter paper changed from yellow (before staining) to orange with Sudan III, orange-red with Sudan IV, and blue-black with Sudan Black B. Floating droplets exposed to Sudan IV changed from yellow (Fig. 1c) to orange (Fig. 1d).

There was no evidence of feeding in any "starved" squid. All squid were accounted for, so no cannibalism occurred. Two of the videotaped squid explored the aquarium walls with their arms for about 10% of the filmed time, and they sometimes made motions as if catching something, but frame-by-frame viewing of those times gave no conclusive evidence of feeding.

In the squid fed the large shrimp, no cecal droplets were seen until 3 h after feeding ended (Table 3). Cecal droplet volume increased on the day of feeding, then decreased, becoming negligible by 3 d later. When cecal drop total

volume was largest (6 h to 2 d after feeding), no drops were seen on either side of the digestive gland (Table 3). Digestive gland droplets were seen more often on the left side (12 times) than on the right side (6 times) (Table 3). Droplets persisted for about 5 d after the squid ate that one shrimp. No droplets were detectable 7 d after feeding (Table 3).

No evidence of droplet expulsion was obtained from squid held in individual bowls. We saw no oil on the water surface, or on paper toweling that was used to wipe the water surface. Droplets disappeared in all four squid sometime between the third and fourth day of starvation.

Discussion

Despite the limited storage or usage of lipids in cephalopods, we show in this work that droplets in the cecal lumen of *Idiosepius notoides* are lipid. To our knowledge, these extracellular droplets have not been reported in any other cephalopod. We believe these droplets are common in this species and that droplets persist for up to 7 d in starved squid as a result of slow absorption, slow expulsion, or both. Our preliminary evidence suggests that expulsion of large drops was not occurring in the laboratory, but expulsion of small droplets could have been undetected.

Because droplets appear to be free and not membrane-bound in the cecal lumen, lipases could have ready access to them. Perhaps droplets can persist in the cecal sac for a week after a meal because no, or few, lipases are consistently present or active there. The digestive gland and cecum both produce various digestive enzymes (Boucaud-Camou and Boucher-Rodoni, 1983), but few studies have demonstrated lipase activity in any cephalopod digestive tract fluids or extracts (Bidder, 1966). Lipases probably occur in cephalopod digestive organs, including those of paralarvae (Boucaud-Camou and Roper, 1995), but oily feces in animals that were fed a diet high in lipids, and the accessibility of lipids in *Octopus vulgaris* for several days after feeding support the conclusion that cephalopod lipid metabolism is "slow and inefficient" (O'Dor *et al.*, 1984). A study of lipase activity in various regions of the digestive tract of *Idiosepius notoides* before, during, and after meals should be informative.

Droplets seemed easier to see in the cecum than in the digestive gland, probably because of the opaque brownish color and tubular structure of the latter organ. In addition, larger droplets may form more readily in the cecum because small droplets might meet and coalesce into larger spheres more easily in the cecal lumen than in the digestive gland, whose structure may interfere with contact between droplets.

We observed movement of small droplets along the length of both the left and right halves of the digestive gland in live squid, but never saw movement of droplets between the two sides. The digestive gland of the congener *I. pygmaeus* is a

Table 2

Summary of evidence supporting the lipid nature of cecal droplets in southern pygmy squid, *Idiosepius notoides*

Criterion	Observation	Consistent with lipid?
Color	yellow	+
Solubility	insoluble in water	+
Density	less than water	+
Paper bag test	translucent mark	+
Sudan stains	sudanophilic	+

Table 3

Number of extracellular lipid drops seen in the cecum and in both sides of the digestive gland of one *Idiosepius notoides* squid over a 7-day period after feeding; the squid was starved in the laboratory for one week before feeding

Time (after feeding)*	Cecum		Digestive Gland			Totals	
	Number of cecal drops	Diameter of cecal drops (mm)†	No. of drops on left side	No. of drops on right side	Location of largest drop‡	Total number of drops	Total cecal drop volume (mm ³ × 10 ⁻²)§
-1 h	0	0	1	1	dg	2	0.0
0 h	0	0	1	1	dg	2	0.0
1 h	0	0	1	1	dg	2	0.0
2 h	0	0	1	1	ldg	2	0.0
3 h	2	nd	1	1	ldg	4	> 0.0
4 h	2	nd	1	0	ldg	3	> 0.0
5 h	2	0.2, 0.3	1	0	ldg	3	1.8
6 h	4	0.2, 0.25, 0.35, 0.45	0	0	cecum	4	8.3
7 h	4	0.1, 0.1, 0.2, 0.55	0	0	cecum	4	9.2
2 d	2	0.1, 0.5	0	0	cecum	2	6.6
3 d	0	0	6	0	ldg	6	0.0
4 d a.m.	3	nd	1	1	dg	5	> 0.0
4 d p.m.	1	0.1	1	0	ldg	2	0.05
5 d a.m.	0	0	1	0	ldg	1	0.0
5 d p.m.	1	0.05	1	0	ldg	2	0.007
7 d	0	0	0	0	—	0	0.0

* 0 h signifies the end of the meal.

† nd = no data.

‡ dg = digestive gland; ldg = left side of digestive gland.

§ Total volume of cecal drops was calculated from the number of drops and their diameter. Digestive gland drops were not measured.

“single unilobed organ” that is “ventrally bilobed with an incomplete dorsal septum” (Semmens *et al.*, 1995). If a digestive gland septum occurs in *I. notoides*, it might explain this apparent separation and separate movement of left and right drops. It does not explain why we saw droplets more frequently on the left side of the digestive gland in the one squid examined repeatedly over a 7-d period (Table 3).

Mantle length seemed irrelevant to either the presence or size of oil droplets, but we could find only two large squid for our study. The fact that the only squid without droplets in Week 1 of starvation was also the only gravid female suggests that further records of reproductive stage *versus* droplets are warranted.

Cephalopods are active carnivores, typically catching crustaceans, molluscs, and fish that are relatively large—often as long as two-thirds of the predators' mantle length (Bidder, 1966; Wells and Clarke, 1996). However, members of *Idiosepius* are the world's smallest cephalopods (Norman and Reid, 2000) and may also be able to feed on small organisms that are not readily apparent to the unaided eye. We observed “nibbling” behavior (Moynihan, 1983) in our squid but no definite feeding during nibbling. Although our “unfed” squid may have fed on small organisms like copepods that were present in the recirculating seawater system, we consider it unlikely that this potential feeding affects any of our conclusions.

Most of the carbon in cephalopod meals is in protein

molecules, and growth in cephalopods is primarily through protein formation (O'Dor *et al.*, 1984). A typical squid body is about 80% muscle, but only 1%–1.5% lipid, and less than 0.4% carbohydrate (O'Dor and Webber, 1986). In line with their proteo-metabolic capabilities, all cephalopod organs, except the digestive gland, are also high in protein and low in lipid (*e.g.*, 10%–17% protein *versus* 1%–2% lipid dry mass in squid mantle, head, testis, or spermatophoric complex [Hochachka *et al.*, 1975; Clarke *et al.*, 1994], and 3% lipid in cuttlefish gonad [Blanchier and Boucaud-Camou, 1984]). The only cephalopod organ containing abundant lipid is the digestive gland (called liver or hepatopancreas in older literature). Content of that organ varies from one species to another: reported values for lipid content are 4%–6% in *Photololigo* sp. (Semmens, 1998; Moltschanivskyj and Semmens, 2000), 8%–11% in *Sepia officinalis* (Blanchier and Boucaud-Camou, 1984), about 30% in *Illex argentinus* (Clarke *et al.*, 1994), and 27%–56% in *Moroteuthis ingens* (Brachi, 1953; Phillips *et al.*, 2001).

Food in a typical cephalopod travels through the buccal mass and down the esophagus to the stomach for initial extracellular digestion, probably aided by salivary and digestive gland enzymes (Boucaud-Camou and Boucher-Rodoni, 1983). Smaller food materials move to the digestive gland or the cecum for further digestion, and wastes travel the intestine to the anus (Bidder, 1966). The cecum, connected to the digestive tract between the stomach and anus,

receives and processes fine particles, has its own sphincter to isolate contents, and is the primary site of absorption from food fluids (Bidder, 1966; Boucaud-Camou and Boucher-Rodoni, 1983; O'Dor and Webber, 1986). Perhaps the cecum of *I. notoides* can absorb dietary lipids, as reported for *Octopus*, *Loligo*, *Sepia*, and *Nautilus* (Bidder, 1966; Boucaud-Camou and Boucher-Rodoni, 1983; O'Dor *et al.*, 1984; Westermann and Schipp, 1998).

In this study, we saw extracellular lipid in two organs: the cecum and the digestive gland. In other cephalopods, the only organ with significant lipid (reported as cellular deposits) is the digestive gland; therefore, it has been considered the only storage site for lipid molecules. These lipid molecules might be oxidized during reproductive maturation or starvation, as in other marine organisms (Voogt, 1983; Boucaud-Camou, 1971, cited in Blanchier and Boucaud-Camou, 1984; Kreuzer, 1984, cited in Castro *et al.*, 1992; Clarke *et al.*, 1994). Besides storing lipids, the digestive gland may be absorptive in some but not all species (Bidder, 1966; Boucaud-Camou and Boucher-Rodoni, 1983). In fact, lipid seen inside digestive gland cells of two squid species appears to be packaged for expulsion, not storage (Semmens, 1998); because expulsion would be energetically wasteful and would make the squid denser, perhaps some lipid is moved to the cecum rather than expelled from the organism. Although very few cephalopods produce and store lipids for buoyancy (Clarke, 1988; O'Dor, 2002), perhaps the cecal lipid in *I. notoides* aides in the support of this small but negatively buoyant cephalopod.

It is clear from our study that retention of extracellular lipid occurs in *I. notoides*; it is less clear whether retention for 7 to 8 days qualifies as "storage." The term storage is used in the literature without reference to length of retention, without evidence of retention *versus* replacement of molecules, and with the implication of future use. Labeling studies may be useful in determining if "storage" of extracellular lipid in the cecum and digestive gland of *I. notoides* is due to slow utilization and/or slow elimination, either or both coupled with the addition of new molecules from the next meal.

During our laboratory study with *I. notoides*, luminal oil droplets disappeared slowly, over a period of days (7–8 days in our field-fed squid; 3–4 days in our mysid-fed squid), not hours or minutes. This slow disappearance of lipid suggests slow absorption, slow expulsion, or both in starved squid. We cannot rule out rapid expulsion of large drops in the field. It is also unknown if this species makes rapid vertical movements in the field. In the laboratory, these squid spent most of their time sitting on aquarium walls or on the undersurface of plastic plants, and movements were mostly horizontal. Anesthetized squid sank to the bottom, indicating that even with lipid droplets in the cecum, these squid are negatively buoyant. Rapid expulsion of large lipid drops by these small cephalopods might pro-

vide a quick increase in negative buoyancy during a dive, and new drops could apparently be formed at the next meal. Our squid could not deep dive in our expulsion study because the water was only a couple of centimeters deep; the water level in our holding tank was about 10 cm deep, and the tank contained no predators that might induce swimming up and down in the water column.

Extracellular lipid droplets have not been previously reported in *Idiosepius* species. Perhaps the drops are specific to *I. notoides*, which is not a well-researched species. Perhaps droplet presence and color vary with lipid content of the field diet; less fatty prey might not lead to droplets, and some prey might lead to paler droplets that are harder to see. Perhaps droplets were overlooked in previous studies of *I. notoides* because tiny droplets in live squid can resemble the yellow chromatophores in size and color, or because the droplets were obscured or extracted by preservatives (*e.g.*, alcohol can turn the cecum opaque white).

We considered using chemical anesthesia on *I. notoides* to help us locate and measure droplets in live squid, but this can move material from organ to organ in the digestive tract of cephalopods (Bidder, 1966). Decapitation and dissection can also cause movement of material between organs (Bidder, 1966). Although movement or breakage of drops due to handling cannot be ruled out in our study, we minimized post-collection handling and confirmed for some squid that droplets were in the cecum before chilling.

We provide preliminary evidence that cecal oil droplets originate from food a few hours after consumption. Although droplets were admittedly less obvious in the digestive gland than in the cecum, the amount of lipid in the cecum at 3–7 h after feeding far exceeded the amount detected in the digestive gland before feeding. Thus, the "new" cecal lipid probably derived from the latest meal.

Cephalopod digestion times (defined as time from food capture to return of stomach and cecum to "hunger condition") include 15–20 h in *Octopus* and *Sepia* and 4–12 h in *Loligo* (Bidder, 1966; Lipiński, 1990). Although cephalopods "digest quickly, convert efficiently, and grow but do not store energy during their 'live fast, die young' lives" (O'Dor and Webber, 1986), we cannot say with certainty that the digestive gland of *I. notoides* was empty after 7 d of starvation. However, because digestive gland lipid in *Octopus* dropped from 0.3% to 0.06% of body weight with a 6-d starvation (O'Dor *et al.*, 1984), 7-d starvation in *I. notoides* may deplete most non-membrane lipid from its digestive gland. The large volume of "new" cecal lipid after feeding, coupled with a week of prior starvation, leads us to conclude that the post-meal cecal lipid seen in *I. notoides* was produced in a few hours from the recent meal. Both the reappearance of lipid drops in the squid fed after a 7-d starvation and the continued absence of drops in all squid starved more than 7 d support this conclusion.

This study describes extracellular lipid droplets in *I.*

notoides but leaves unanswered whether these tiny cephalopods expel the material over time, whether they are metabolically capable of obtaining energy from the lipid, and whether the drops confer a buoyant advantage. The fact that lipid droplets did not disappear until the eighth or ninth day of starvation in field-fed animals suggests that these squid may use the droplets as an energy source. However, slow expulsion of the lipid as a dietary waste cannot yet be ruled out.

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